PATENT
Attorney Docket No. 161192

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Saavedra et al.	
Serial No. 08/837,812	Group 1615
Filed: April 22, 1997	Examiner P. Kulkosky
For: Biopolymer-Bound Nitric Oxide-Releasing Compositions, Pharmaceutical Compositions Incorporating Same and Methods of Treating Biological Disorders Using Same	

DECLARATION OF JOSEPH A. HRABIE

Assistant Commissioner For Patents
Washington, D.C. 20231

Dear Sir:

Dr. Joseph A. Hrabie hereby declares and states as follows:

1. He is presently employed as Scientist in the Chemical Synthesis and Analysis Laboratory, SAIC Frederick, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland.

2. He received his Bachelor of Science from Queens College, Flushing, New York, in 1975 and his Ph.D. in Organic Chemistry from the City University of New York, New York, in 1981. He has been engaged in the research and development of new organic compounds and in the synthesis of organic compounds since 1981. He has authored over twenty articles relating to organic synthesis and he is the inventor of numerous patents. A copy of his curriculum vitae is attached hereto as Exhibit A.

3. He has read and he understands U.S. patent application Serial No. 08/837,812 (hereinafter referred to as the "'812 application"), including claim 1 of that application.

4. He has also read and he understands the Advisory Action issued by the U.S. Patent and Trademark Office on November 20, 1998.

5. In his opinion, it would not require a synthetic organic chemist to engage in an unreasonable amount of laboratory experimentation in order to synthesize nitric oxide-releasing diazeniumdiolate derivatized biopolymers, as described and claimed in the '812 application.

6. In his opinion, based on his experience in the synthesis of organic compounds, the description in the specification of the '812 application of the use of a diazeniumdiolate of the formula X-[NONO]- or -[NONO]-X to derivatize the biopolymeric backbone of an oligonucleotide, a nucleic acid, an antibody, or a tissue specific, cell specific or tumor specific antibody, or a portion such an antibody, a protein containing a recognition sequence for a receptor-ligand interaction favorable to tumor attachment, an anti-chemostatic agent or a hormone enables one skilled in the art to form a diazeniumdiolated biolymer capable of releasing nitric oxide.

7. The specification teaches the skilled organic chemist that the reaction allowing the $N_2O_2^-$ functional group to attach to the biopolymer occurs at the C-terminus and at the N-group of an oligopeptide or protein backbone. See, for example, p. 6, lines 35-39 and p. 7, lines 1-5; p. 8, lines 3-34; p. 15, lines 10-29; p. 16, line 17; and p. 17, line 12. In particular, "linking group" X or X' in the disclosure at p. 6, lines 35-39 and p. 7, lines 1-5 identifies a well-known subset of organic chemical reagents that have been developed for the purpose of attaching functional groups to polymers. Linking groups are familiar to peptide chemists, they have been summarized in numerous reference texts on the subject (see, for example: "Techniques in Protein Modification," R.L. Lundblad, 1995, CRC Press, Boca Raton, Florida - Exh. B) and

they are commercially available from many companies including, for example, Pierce Chemical Company (Rockford, Illinois), whose catalog also summarizes linker chemistry in detail (Exh. C). Linking group chemistry is now considered standard methodology in peptide science, and can be used to modify biopolymers as described in the '812 specification.

8. The Examples of the specification further illustrate the enabling disclosure. In Example IV, the preparation of the bis(nitric oxide) adduct of L-prolyl-L-leucylglycinamide is exemplified. In Example V, the attachment of a nucleophilic center to a protein that does not contain a nucleophilic center that will readily react with NO is exemplified. In Example VI, the attachment of a preformed diazeniumdiolate containing a nucleophilic nitrogen atom to the C-terminus of a peptide, polypeptide or protein is exemplified. Examples V and VI, in particular, teach the application of the standard reagent dicyclohexylcarbodiimide (DCC) to the preparation of the compounds claimed and thus one skilled in the art would immediately recognize and be able to repeat these procedures with any biopolymer. In his opinion, these Examples thus teach an organic synthetic chemist that biopolymers can be derivatized with a diazeniumdiolate.

9. In addition, the syntheses described in the specification of the '812 application have been used to prepare nitric oxide-releasing diazeniumdiolate derivatives of nucleotides. For example, the paper "Piperazine as a Linker for Incorporating the Nitric Oxide-Releasing Diazeniumdiolate Group into Other Biomedically Relevant Functional Molecules," by Saavedra et al., submitted to J.Org.Chem., attached hereto as Exh. D, describes nitric oxide-releasing nucleotides. Saavedra et al. report that the halogen of 6-chloropurine riboside may be displaced by the nitrogen of a diazeniumdiolated piperazine to form a nitric oxide-releasing nucleoside analog (see compound 9g of Exh. D). In his

opinion, this synthesis shows that the two major purine bases found in nucleosides, i.e., adenine and guanine, can be derivatized with a diazeniumdiolate to form nitric oxide donors in accordance with the disclosure of the specification of the '812 application.

10. Since an oligonucleotide is a compound in which several mononucleoside units are joined together through phosphodiester linkages, and the experiments described above demonstrate that purine bases found in nucleosides can be derivatized with diazeniumdiolates to form NO-releasing nucleosides, in his opinion, the disclosure of the specification of the '812 application teaches that any oligonucleotide can be derivatized with a diazeniumdiolate.

11. In his opinion, the disclosure of the '812 application also enables the skilled organic chemist to derivatize nucleic acids with nitric oxide-releasing diazeniumdiolates because nucleic acids are extremely large oligonucleotide molecules, and, like synthetic oligonucleotides, include at least one purine base that can be derivatized.

12. Protein derivatives which release nitric oxide are disclosed in "Conversion of Proteins to Diazeniumdiolate-Based Nitric Oxide Donors," by Hrabie et al., submitted to Bioconjugate Chem. (attached hereto as Exh. E). The derivatization of the ε-amino groups of the amino acid lysine with a nitric oxide diazeniumdiolate (compound 5) is accomplished by the methods disclosed in the '812 application using a "linking group" as described, for example, at p. 6, line 38 and p. 7, line 2.

13. Since all antibodies are larger proteins and will almost invariably contain one or more lysines, in his opinion, antibodies would generally be derivatizable with diazeniumdiolates. In his opinion, the presence of any particular molecular recognition sequence elsewhere in a long

chain peptide would not reasonably be expected to interfere with the derivatization reaction.

14. In his opinion, the '812 application has enabled the skilled organic chemist to make a nitric oxide-releasing diazeniumdiolated biopolymer. Such knowledge and information can be extended to a wide variety of biopolymers and diazeniumdiolates to provide numerous species of diazeniumdiolated biopolymers.

15. He hereby declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

3/31/99
Date

Dr. Joseph A. Hrabie

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LIST OF EXHIBITS
Declaration of Joseph A. Hrabie
U.S. Serial No. 08/837,812
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A Joseph A. Hrabie Curriculum Vitae

B "Techniques in Protein Modification"
R.L. Lundblad, 1995, CRC Press

C Pierce Chemical Company Catalog

D Saavedra et al., "Piperazine as a Linker for Incorporating the Nitric Oxide-Releasing Diazeniumdiolate Group into Other Biomedically Relevant Functional Molecules," submitted to J.Org. Chem.

E Hrabie et al., "Conversion of Proteins to Diazeniumdiolate Based Nitric Oxide Donors," submitted to Bioconjugate Chem.